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Evaluation of Three Portable Samplers for Monitoring Airborne Fungi

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Airborne fungi were monitored at five sample sites with the Burkard portable, the RCS Plus, and the SAS Super 90 air samplers; the Andersen 2-stage impactor was used for comparison. All samplers were calibrated before being used simultaneously to collect 100-liter samples at each site. The Andersen and Burkard samplers retrieved equivalent volumes of airborne fungi; the SAS Super 90 and RCS Plus measurements did not differ from each other but were significantly lower than those obtained with the Andersen or Burkard samplers. Total fungal counts correlated linearly with *Cladosporium* and *Penicillium* counts. *Alternaria* species, although present at all sites, did not correlate with total count or with amounts of any other fungal genera. Sampler and location significantly influenced fungal counts, but no interactions between samplers and locations were found.

Assessing occupational exposure to bioaerosols requires reliable devices with which to measure viable airborne microorganisms (13). Several sampling devices are available commercially for identifying and enumerating airborne microorganisms (1, 5–7, 9–11, 14). The Andersen six-stage and two-stage viable (microbial) particle-sizing samplers are widely considered the samplers of choice for enumerating viable microorganisms. However, their large size and dependence on line current (external power supply) have limited their use in remote locations. Jensen et al. (9), in evaluating the relative sampling efficiencies of eight bioaerosol samplers under controlled conditions in a horizontal bioaerosol chamber, found the Andersen six-stage, Andersen one-stage, and Ace AGI-30 samplers to be the best for recovering aerosols of free microorganisms (9). Buttner and Stetzenbach (4) later monitored aerosols in an experimental room to select sampling methods for retrieving fungal spores and to determine the effect of human activity on air sampling. In that study, the Andersen six-stage viable impactor and the Burkard spore trap retrieved the greatest numbers of fungal spores (4). This report focuses on the relative efficiencies of the Burkard portable, RCS Plus, and SAS Super 90 air samplers, none of which has been evaluated thus far. The Andersen two-stage impactor was used as the reference sampler.

Air samples (n = 240, 100 liters each) were collected as follows: 12 samples × 4 samplers × 5 locations. Three areas were sampled in a mid-sized building that housed offices and laboratories (an extended hallway, containing vending machines and two doors opening to the outdoors; an electrical room; and a mechanical room); two additional areas (a living room and a garage) were sampled at an apartment building. All four samplers were operated simultaneously at each site, at a distance of 1 m apart; samples were collected at different times of day on several days.

Air samplers. The Andersen two-stage viable impactor (Graseby Andersen, Atlanta, Ga.) draws air at 28.3 liters/min. Each stage has 200 holes, 1.5 mm diameter in the first stage and 0.4 mm in the second. The efficiency curves of impactors such as this can be characterized by the Stokes number, Stk_{50}, which describes 50% collection efficiency (9). Use of this number is equivalent to assuming that the masses of particles larger and smaller than the cut diameter (d_{50}) are equal. Hence, the d_{50} is the aerodynamic diameter, above which the collection efficiency of the impactor approaches 100% (7). The d_{50} for the first and the second stages of the Andersen device are 8.0 and 0.95 μm, respectively (1). The Andersen sampler was calibrated with a rotameter that had been precalibrated with a dry gas meter, which in turn had been calibrated with a Brook's Bell Prover (primary standard) by the Johnson Space Center Calibration Laboratory. The Andersen sampler, each stage equipped with a petri dish containing agar medium prepared according to the manufacturer's specifications (2), was connected to a standard rotameter with plastic tubing, and the flow rate was set at 28.3 liters/min.

The Burkard portable air sampler for agar plates (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, United Kingdom) operates on the same principle as the Andersen sampler but is powered by a rechargeable nickel cadmium battery. Its sieve plate has 100 holes of 1 mm diameter; a 90-mm petri dish is placed below the plate to collect airborne microorganisms. The sampler can be switched on and off manually or automatically with a built-in timer. The Burkard sampler was calibrated by evacuating a known quantity of air from a plastic bag and measuring the elapsed time (8). The flow rate, taken as the mean of 20 readings, was 28.3 liters/min (n.b., manufacturer's stated rate is 10 or 20 liters/min). The d_{50} values for the Burkard air sampler, calculated by the authors, was 2.56 mm.

The RCS Plus (Biotest Diagnostics Corp., Denville, N.J.), a centrifugal impactor, operates on an entirely different principle than the Andersen and the Burkard devices. Air samples are impacted onto isolation medium contained in plastic strips (with 34 wells approximately 1 cm^2 each). The d_{50} for this air sampler (6 mm) also was calculated by the authors. Air flow was calibrated with a digital flywheel anemometer, supplied by the manufacturer, as follows. The anemometer sensor was fitted onto the protective cap of the sampler, an agar strip was loaded, and a connecting cable was screwed to the display unit.

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TABLE 1. Counts of airborne fungi measured at five locations with four air samplers[a]

Location and device	Count ($\log_{10}$ CFU/m^3)			
	Total fungi	*Cladosporium* spp.	*Penicillium* spp.	*Alternaria* spp.
Apartment				
Andersen	2.88 (0.06)	2.50 (0.08)	1.67 (0.20)	0.73 (0.19)
Burkard	3.03 (0.08)	2.31 (0.11)	1.96 (0.16)	0.41 (0.14)
SAS Super 90	2.62 (0.08)	2.20 (0.07)	1.65 (0.15)	0.19 (0.13)
RCS Plus	2.47 (0.07)	2.12 (0.08)	1.38 (0.20)	0.08 (0.08)
Garage				
Andersen	2.99 (0.12)	2.51 (0.14)	1.35 (0.08)	0.73 (0.19)
Burkard	3.10 (0.11)	2.51 (0.15)	0.76 (0.20)	0
SAS Super 90	2.58 (0.07)	2.29 (0.07)	0.38 (0.16)	0
RCS Plus	2.57 (0.08)	2.22 (0.08)	0.73 (0.20)	0.08 (0.08)
Vending area				
Andersen	1.92 (0.07)	1.60 (0.08)	0.62 (0.19)	0.16 (0.11)
Burkard	2.11 (0.09)	1.48 (1.23)	0.93 (0.17)	0.33 (0.14)
SAS Super 90	1.53 (0.17)	1.08 (1.21)	0.50 (0.18)	0
RCS Plus	1.69 (0.16)	1.17 (1.22)	0.35 (0.15)	0.08 (0.08)
Electrical room				
Andersen	2.28 (0.08)	2.02 (0.10)	0.83 (0.22)	0.21 (0.14)
Burkard	2.20 (0.13)	1.91 (0.19)	0.97 (0.18)	0
SAS Super 90	1.89 (0.11)	1.68 (0.15)	0.35 (0.15)	0.25 (0.13)
RCS Plus	1.94 (0.12)	1.55 (0.23)	0.51 (0.18)	0
Mechanical room				
Andersen	2.98 (0.04)	2.78 (0.06)	1.48 (0.07)	1.19 (0.23)
Burkard	3.01 (0.11)	2.78 (0.13)	1.04 (0.19)	0.16 (0.11)
SAS Super 90	2.68 (0.07)	2.56 (0.08)	1.06 (0.16)	0.19 (0.13)
RCS Plus	2.69 (0.05)	2.54 (0.07)	0.73 (0.10)	0.10 (0.10)
Total (all locations)				
Andersen	2.61 (0.04)	2.28 (0.08)	1.19 (0.07)	0.61 (0.05)
Burkard	2.69 (0.04)	2.20 (0.11)	1.13 (0.07)	0.18 (0.05)
SAS Super 90	2.26 (0.04)	1.92 (0.07)	0.79 (0.07)	0.12 (0.05)
RCS Plus	2.27 (0.04)	1.96 (0.08)	0.74 (0.07)	0.07 (0.05)

[a] Data are means (standard error) of 12 samples.

The mean flow rate from three runs was 50 liters/min. The surface air sampler (SAS) Super-90 (PBI International, Milan, Italy) aspirates air at a fixed speed for variable periods through a 219-hole cover and onto a 55-mm RODAC contact plate (PBI International). The d_{50} (calculated by the manufacturer) for the SAS Super 90 was 2.0 to 4.0 μm. The SAS Super 90 was calibrated by bag evacuation, like the Burkard sampler. The mean flow rate from 20 repetitions was 85 liters/min. (The factory calibration was stated as being 90 liters/min.)

Sample collection and data analysis. The Andersen and Burkard samplers were set to run for 3.5 min (100 liters); the SAS Super 90 and RCS Plus samplers were set to collect 100 liters of air. The DG-18 medium used for the air samplers was prepared as follows. To 500 ml of distilled water, 15.75 g of dichloran glycerol agar base (Oxoid Ltd., England) was added and the mixture was heated to dissolve the contents. Then, 100 g of glycerol (analytical reagent grade) was added, and this mixture was sterilized by autoclaving it to 121°C (15 lb/in^2 of pressure) for 15 min. The mixture was allowed to cool to about 50°C, 50 mg of SR-78 chloramphenicol supplement was added and the solution was mixed thoroughly. Aliquots of this medium then were added to either a 55-mm RODAC contact plate (10 ml, for the SAS Super 90) or 90-mm plastic petri dishes (20 ml for the Andersen and 27 ml for the Burkard sampler). Commercially available DG-18 strips (Biotest Diagnostics) were used with the RCS Plus. After samples were collected, the agar plates or strips were removed from the samplers, covered, and incubated at 25°C for 5 days. Colonies of filamentous fungi were identified to genus level on the basis of colonial and microscopic morphology. The number of fungal colonies on each plate were counted, adjusted for multiple impaction using a "positive hole" conversion table as needed (12), and then converted to CFU/m^3 of air. The conversion factor for each sampler depended on the number of holes, i.e., 200 for the Andersen, 219 for the SAS Super 90, and 100 for the Burkard. A positive hole table for the Burkard device was generated from the formula $P_r = N[1/N + 1(N - 1) + 1/(N - 2) + 1/(N - 3) + \ldots 1/(N - r + 1)]$, where P_r is the expected number of viable particles to produce r positive holes (where r is the number of colonies or CFU observed on the specimen plate, equal to the number of positive holes), and N is the total number of holes per stage (12).

To approximate a normal distribution, numbers of CFU per cubic meter were converted to $\log_{10}$ units, and these transformed numbers were used for a $12 \times 4 \times 5$ analysis of variance (ANOVA) to evaluate the number of counts per sampling session ($n = 12$) as a function of sampling device ($n = 4$) and sampling location ($n = 5$). Transformed data were used for all statistical analyses. A randomized complete block design was used. Locations were considered distinct among themselves and were used to control the inherent variability in data. No interaction was found between air sampler and location. The analysis of variance results made use of the pooled variance of data from different samplers. The least significant t test was used for comparisons among the four air samplers; this test is viewed to be the most appropriate here since the four air samplers formed two distinct groups (Andersen plus Burkard and RCS Plus plus SAS Super 90; see below). The two groups significantly differed in retrieving the fungal counts, but no significant differences were present within a group.

The ANOVA results from all the data values revealed significant differences among the sampling devices with regard to total fungal count, *Cladosporium* count, and *Penicillium* count ($P < 0.0001$) but not for *Alternaria* count (Table 1). Predictably, location was associated with variability in all fungal genera ($P < 0.0001$). However, no interaction was found between location and sampling device, indicating that the samplers performed consistently at all locations. With regard to ability to retrieve total fungal propagules, the Andersen and Burkard samplers were comparable in all sampling sessions at all sampling locations (Fig. 1). Similarily, no significant differences were noted in their abilities to recover *Cladosporium*, *Penicillium*, and *Alternaria* species. The Burkard impactor retrieved slightly more total fungi (CFU per cubic meter) than the Andersen at all locations except the electrical room; however, the mean difference was not statistically significant ($P = 0.23$). Box-plot comparisons of the four samplers in $\log_{10}$ units of total fungi, *Cladosporium* species, and *Penicillium* species showed that the data were nearly normal (data not shown). Total fungal counts (CFU per cubic meter) collected by the RCS Plus and SAS Super 90 were comparable to each other but were significantly less than those of the Andersen and Burkard samplers ($P < 0.0001$). Figure 2 shows the 95% confidence intervals of mean fungal counts for the four air samplers used in the study. The Andersen sampler recovered slightly more propagules of *Cladosporium* species at all locations except the garage, but this difference was not different statistically from that of the Burkard sampler. The Burkard sampler recovered more *Penicillium* species than the Andersen sampler at three of the five locations. Greater numbers of *Alternaria* species were collected by the Andersen sampler than by the Burkard sampler at four of the five sites. The SAS Super 90 and RCS Plus retrieved fewer total fungi and *Cladosporium*,

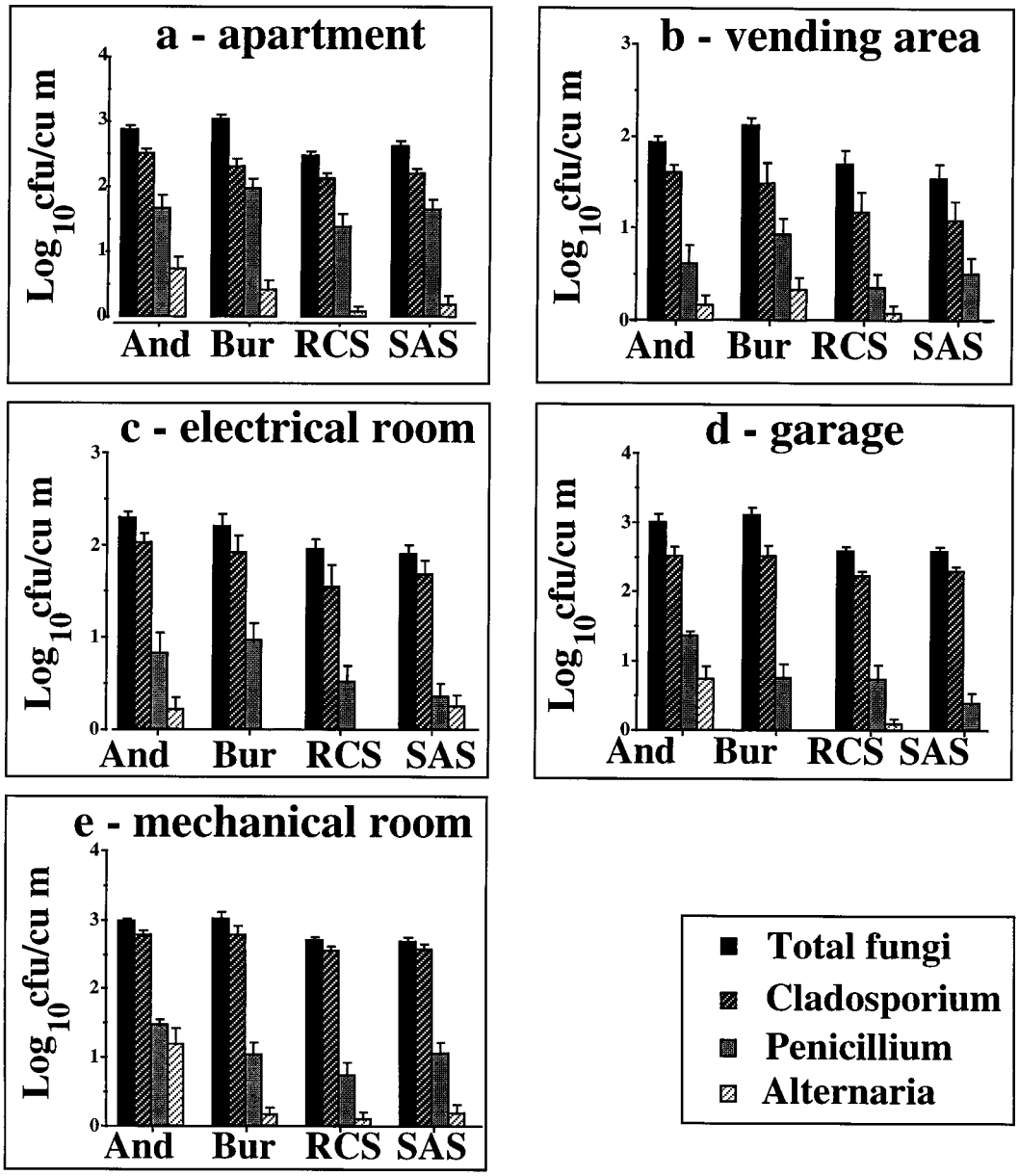

FIG. 1. Fungal recovery from five sampling sites (three occupational and two residential); bar heights represent mean of 12 samples ± 1 standard error.

Penicillium, and *Alternaria* species than the Burkard and Andersen samplers at all sampling sites.

Cladosporium, *Penicillium*, and *Alternaria* species were predominant, but *Aspergillus* spp., *Aureobasidium* spp., *Curvularia* spp., *Drechslra* spp., *Epicoccum* spp., *Fusarium* spp., yeasts and unidentified hyphomycetes were present occasionally. A strong, linear relationship was present between total fungal count and *Cladosporium* count (partial correlation coefficient, 0.66; $P < 0.0001$) and a less strong relationship was present between total fungal and *Penicillium* counts (partial correlation, 0.30; $P < 0.0001$). The presence of *Alternaria* species was not correlated with total fungal count or with any of the other fungal genera (partial correlation coefficient, *Alternaria* spp. versus total fungi, 0.0174; *Alternaria* spp. versus *Cladosporium* spp., 0.05; and *Alternaria* spp. versus *Penicillium* spp., 0.06).

The Andersen sampler was comparable to the Burkard sampler in retrieving airborne fungi. The other two samplers, the RCS Plus and the SAS Super 90, formed a second group with comparatively lower recovery of airborne fungi counts. Substantial overlap was observed at 95% confidence intervals for the Andersen and Burkard and for the RCS Plus and SAS Super 90 devices, for $\log_{10}$ transformed data for total fungi, *Cladosporium* counts, and *Penicillium* counts (Fig. 2 a to d).

The results reported here demonstrate for the first time that the Burkard portable air sampler is comparable to the Andersen two-stage impactor for the collection of total fungi, *Cladosporium* species, or *Penicillium* species. Both the Andersen and Burkard samplers can collect particles larger than 3 μm efficiently; the smallest airborne fungal spores usually range from 2 to 10 μm in diameter. The theoretical d_{50} for the SAS Super 90 (2 to 4 μm) suggests that that device would recover fewer fungal spores than the Andersen or Burkard samplers. How-

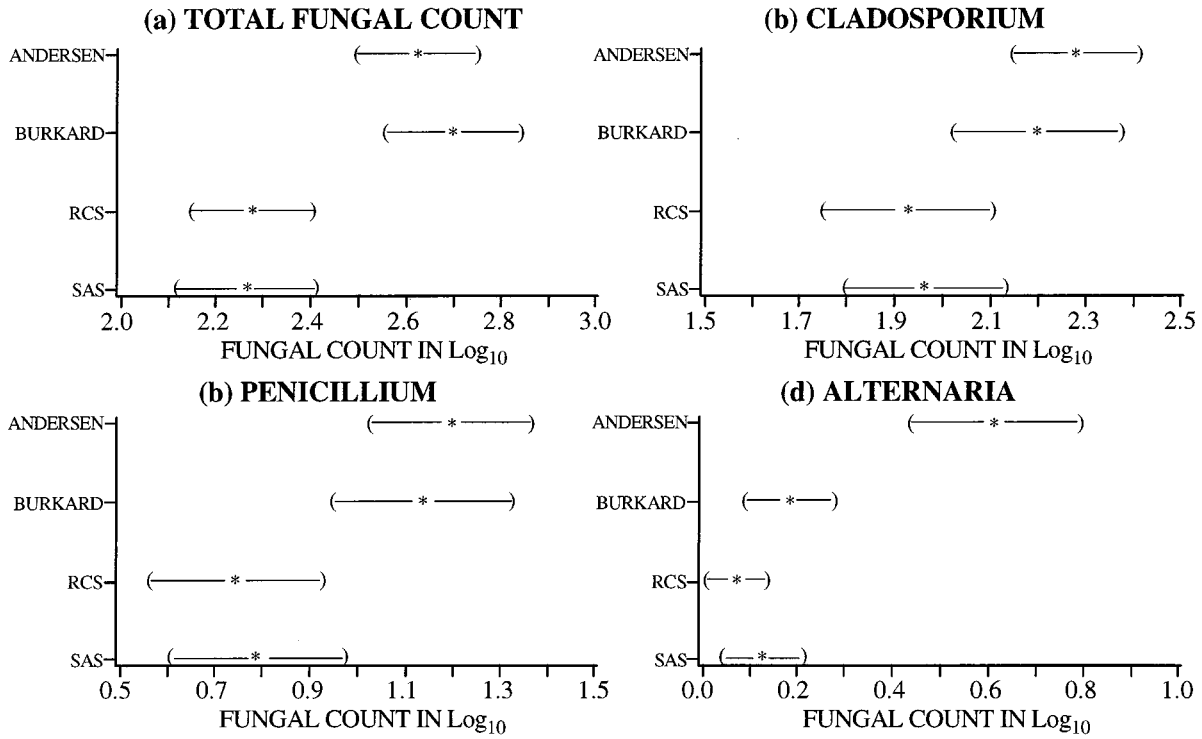

FIG. 2. Confidence intervals (95%) for comparing the efficiency of the 4 samplers in retrieving mean total fungi (panel a), *Cladosporium* spp. (panel b), *Penicillium* spp. (panel c), and *Alternaria* spp. (panel d).

ever, none of these four air samplers can recover viable airborne particles without some inactivation or loss, either during or after sampling. Consequently, the efficiency of any air sampler will vary depending on the device used and the nature of the aerosol sampled (3). In conclusion, the Burkard device seems to be an excellent alternative to the Andersen unit for use in settings where line current is not available and where the size and weight of the sampler must be minimized.

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